

ings was validated for the new, engineered 293-F cell lines. A, Representative confocal microscopy images of stable suspension adapted human embryonic kidney 293 (293-F) cell lines—wild type (w.t.), or stably expressing the Mucin-0, Mucin-135, or Mucin-270 biopolymer. Images show the cell membrane (shown in blue, CF633 Wheat Germ Agglutinin, WGA), O-glycans covalently attached to the Mucin-135 and Mucin-270 biopolymers (shown in red, CF568 Peanut Agglutinin, PNA), and green-fluorescent protein (shown in green, GFP) which is co-expressed on the plasmid with the Mucin-0, Mucin-135 and Mucin-270 biopolymer. B, Representative flow cytometry histograms showing the polydisperse population of biopolymer expressing cell lines compared to w.t. cells, y-axis is scaled to show the population distribution of GFP positive cells. >50,000 cells per histogram. C, Quantification of the percent of cells which are GFP positive for each cell line. Cells with GFP signal above the gray line in FIG. 2B were considered GFP positive. Mean and S.D. are shown, >50,000 cells per sample, n=4. D, Representative immunoblot (left) and lectin blot (right) of whole cell lysates for each generated stable cell line compared to w.t. cells, n=3. E, Viable cell concentration determined by hemocytometer counting with trypan blue exclusion, n=3. F, GFP signal of Mucin-270 cells after induction of expression at t=0 hr, measured by flow cytometry, n=3, >15,000 cells per sample. G, Agarose gel showing polymerase chain reaction (PCR) product of Mucin-270 gene from DNA extracted from non-transfected cells (Mock), w.t. cells transiently transfected (Transient), or cells with the Mucin-270 gene incorporated in the genome and cultured for 2 months (2 mo.) or 12 days (12 d) after gentamycin selection. Star indicates the predicted molecular weight of Mucin-270 PCR product. #1 and #2 are biological replicates. Mean and S.D. shown, ns—not significant.

[0031] FIG. 14: Biopolymer Coatings Reduced Cell Aggregation. Genetically-encoded biopolymer coatings of Mucin-135 and Mucin-270 size reduce cell aggregation in suspension cell culture. A, Representative phase contrast images for w.t. and biopolymer cell lines. Images were for cells grown at a concentration of $3.8 \pm 0.7 \times 10^6$ cells/mL at 72 hr post-induction. B, Quantification of the fraction of cells in various cluster sizes from phase contrast images such as those shown in FIG. 3A, 3 biological replicate samples, 2 technical replicate samples, 3 images analyzed per sample, samples (further discussion of replicates in Materials and Methods section). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means. C, Quantification of the fraction of cells which are in clusters of various sizes from phase contrast images such as those shown in FIG. 3A. Mean and S.D. are shown. D, Ripley's K function versus distance calculated for the cell distribution acquired from phase contrast images such as those shown in FIG. 3A. Mean and S.E.M. are shown, replicates described in FIG. 3B. ns—not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

[0032] FIG. 15: Mucin-270 Reduced Aggregation in High Calcium Culture Media. The Mucin-270 cell line out-performs commercial anti-clumping solution in highly aggregating conditions. A, Image of Mucin-270 and w.t. cultures grown in media with 2 mM CaCl_2 ($+\text{Ca}^{2+}$). Mucin-270 expression significantly decreases cell aggregation, even compared to commercially available anti-clumping reagent

(+anti-clump). B, Quantification of the concentration of w.t. or Mucin-270-expressing cells in suspension for control cultures with no treatment (null), with the addition of commercial anti-clumping reagent (+anti-clump), with the addition of 2 mM CaCl_2 ($+\text{Ca}^{2+}$), or with both anti-clumping reagent and 2 mM CaCl_2 (+anti-clump+ Ca^{2+}). Statistical comparison is to null condition for each cell line. Mean and S.D. are shown, n=3. ns—not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

[0033] FIG. 16: Biopolymer Coating Enhanced Resistance to Shear Stresses. Expression of the stably incorporated biopolymers protects cells from shear stresses. A, Schematic representation of the experimental setup for shearing cells. Briefly, cells were sheared by flowing through a 500 m Teflon tube under a constant applied force of 1 kg in gravity before being analyzed by flow cytometry with a live/dead cell stain. B, Quantification of the fraction of dead cells after shearing the cells for the w.t. and biopolymer cell lines, Mean and S.E.M. are shown, >50,000 cells measured for each population, n=6. ns—not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

[0034] FIG. 17: Biopolymer Coated Cells can be Transfected. Transfection was determined for the biopolymer coated cell lines by transfection with a cytoplasmic red-fluorescent protein (RFP). A, Quantification of the number of cells for w.t. and biopolymer coated cells transiently transfected with cytoplasmic RFP. The count of transfected cells was normalized to the count of w.t. cells transfected per experiment to account for variable transfection efficiency between replicate transfections. >50,000 cells measured for each population, n=3. B, Representative flow cytometry histogram showing the distribution of expression among transfected cell populations. The peak to the left of the gray line, centered around zero, represented the non-transfected population for each cell line which is further validated by the overlapping histogram of non-transfected w.t. cells (w.t.-null). C, Quantification of the geometric mean of RFP for positively transfected cells from B. Mean and S.D. shown, ns—not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

[0035] FIG. 18: Mucin-270 cells Produced Comparable Levels of Recombinant Protein Expression. Quantification of secreted, recombinant RFP from media supernatant of w.t. or Mucin-270-expressing cultures transiently transfected with secreted RFP, n=3. Mean and S.D. shown, ns—not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

[0036] FIG. 19: Additional data to accompany FIG. 14 acquired 24 hr prior. A, Quantification of the fraction of cells in various cluster sizes from phase contrast images such as those shown in FIG. 3A. Cells are grown at $3.2 \pm 0.7 \times 10^6$ cells/mL for 48 hr for all panels. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means. B, Quantification of the fraction of cells which are in clusters of various sizes from phase contrast images such as those shown in FIG. 3A. Mean and S.D. are shown. C, Ripley's K function versus distance calculated for the cell distribution acquired from phase contrast images such as those shown in FIG. 3A. Mean and S.E.M. are shown, replicates described in FIG. 3B, n=3. ns—not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.